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Evaluation of Salivary Cotinine Level in Tobacco Chewers - A Case-Control Study

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Abstract: ***Aim and objective: To compare the salivary cotinine level in tobacco chewers and non chewers and to corelate cotinine level with the duration of tobacco chewing habit. Methodology: The study was conducted in 94 subjects, divided into two groups, control Group and study group, with 47 participants in each group. The salivary samples were collected from all the participants and cotinine concentration was measured using competitive Elisa method. Result: The mean salivary cotinine levels in group 1 and group 2 was 6.649 and 92.8040 respectively. When mean values were compared between the groups the values were found to be statistically highly significant, and study also showed there is no correlation between the salivary cotinine level and duration of the tobacco chewing habit. Conclusion: The result of our study showed the increased levels of cotinine in chewers as compared to non-chewers. No association was noted between cotinine levels and duration of chewing. Salivary cotinine level proved to be a useful biomarker of recent tobacco use and can be used in epidemiological studies and tobacco cessation programs.***

Keywords: Cotinine, Tobacco chewers, Biomarker, Elisa

1. Introduction

Tobacco is derived from two main species *Nicotiana tabacum* and *Nicotiana rustica*. Its consumption is the chief cause of death in many developing countries[1]. In India, since ancient times, the tobacco consumption is followed in various parts in various forms and patterns[2], mainly smoking and smokeless forms[3].

Smokeless tobacco is the non-combusted form of tobacco that can be taken in orally or nasally. Oral smokeless tobacco products are placed in the oral cavity and the contents are either sucked or chewed[4]. More than 40 forms of smokeless tobacco are available and consumed globally. *Nicotine is* the most important ingredient from tobacco leaves. It is a volatile alkaloid, and is one of the most addictive and stimulant drugs. Nicotine affects all the organs, but mainly, it binds to a central nervous system receptor and increases brain dopamine levels making it an addictive agent. In case of Smokeless tobacco, nicotine is directly absorbed into the body through the mucous membranes in the mouth or nose.

Cotinine is the major metabolite of nicotine and has become the standard marker of nicotine exposure[6], with a long half life of 15 to 19 hours. This pharmacologically inactive compound is slowly cleared from the body and is specific to Tobacco[7]. Nicotine is not considered a valid marker due to its relatively short half-life (approximately two hours).

Most of the estimation of tobacco use in youth are based on self-reports, but biochemical validation is important because rejection and underestimation are common practices especially among young population.

Therefore this study was aimed to evaluate the salivary cotinine level in tobacco chewers and non chewers, and to compare the level with duration of tobacco chewing habit.

2. Materials and Methods

A case-control study was carried out after receiving ethical clearance from the institutional ethical committee; Samples were collected from the out patients, after obtaining informed consent. On the basis of convenience sampling method, sample sizes of 94 were included as per Inclusion and exclusion criteria.

Patients between the age of 18 to 60 years with tobacco chewing habit were included. Patients who were tobacco smokers, having any other substance abuse, systemic illness, on nicotine replacement therapy or on any medications were omitted from the study.

The subjects were divided into Group 1 (with no tobacco chewing habit) and Group 2 (with tobacco chewing habit). Each group had 47 patients.

Unstimulated saliva was collected through "Spit Technique" and transferred to laboratory for analysis. Samples were centrifuged in micro-centrifuge tubes at 3000rpm for 10 minutes and the supernatant collected was stored at -20°C. For processing, the samples were taken out from the deep freezer and brought to room temperature. Cotinine Direct Elisa kit (CALBIOTECH) was used to analyse the samples, absorbance was read on Elisa reader (Thermoscietific, Multi scan sky) using Skan. It software at 450nm

3. Results and Observations

Statistical analysis was done using independent t-test to compare cotinine concentration between the groups and p-value less than <0.001 was considered as significant and the pearson's correlation to estimate the correlation between duration of tobacco chewing and cotinine concentration.

Table 1 shows the demographic data of both the groups. The mean age of the control group was 30.51 years with a standard deviation of 13.917, and for the case group it was 43.2 years with a standard deviation of 7.354. The mean duration of chewing was 11.75 with a standard deviation of 10.266.

Table 2 shows the gender distribution. In control group 44 females (93.6%) and 3 (6.4%) were males and in case group only 3 (6.4%) were females and 44 (93.6%) were males

Table 3 shows the correlation between the duration of tobacco chewing and cotinine concentration. It was found to be -.071, with p-value 0.633 , shows there is no significant correlation between duration of tobacco chewing and cotinine concentration

Table 4 shows Comparison of continine concentration between case and control group. In control group mean cotinine concentration is 6.649 with a standard deviation of 3.12261, and in case group mean cotinine concentration was 92.8040 with a standard deviation of 3.12261.

There is significant difference in mean cotinine concentration between the groups with p <0.001. Cotinine concentration in cases are more compared to control group

Graph 1 shows there is significant difference between cotinine concentration between groups.

Table 1: Demographic data

	Group	N	Mean	Std. Deviation
Age	Case	47	43.26	13.917
	Control	47	30.51	7.354
duration of tobacco chewing	Case	47	11.7500	10.26695

Table 2: Gender Distribution

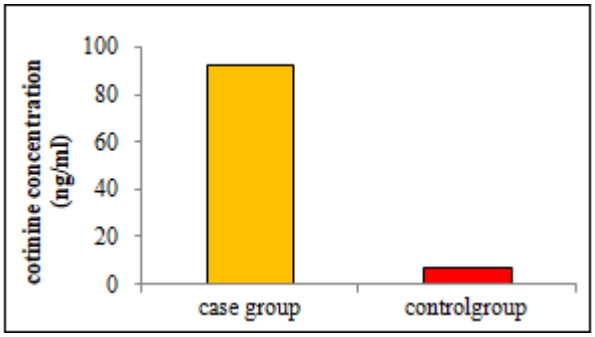
	Gender	
	Female	Male
Case	3	44
	6.4%	93.6%
control	44	3
	93.6%	6.4%

Table 3: Correlation between the duration of tobacco chewing and continine concentration

		Cotinine concentration, ng/ml
duration of tobacco chewing	Pearson Correlation	-.071
	p-value	.633
	N	47

Table 4: Comparison of continue concentration between case and control

	G	N	Mean	Std. Deviation	p-value
Cotinine concentration ng/ml	Case	47	92.8040	4.60608	<0.001
	Control	47	6.6498	3.12261	



Graph 1

4. Discussion

Specific estimation of tobacco consumption is an important concern and in most circumstances it is determined by the questionnaires. The validity of this self reporting is questionable. So tobacco exposure can be assessed by evaluating its biomarkers from body fluids[1]. A number of biochemical markers have been used to identify tobacco use, including measures based on thiocyanate, carbon monoxide, nicotine, cotinine etc.

Nicotine through various pathways gets metabolized to a number of metabolites by the liver. Six primary metabolites of nicotine have been identified. The most important metabolite is Cotinine and in humans about 70–80% of nicotine is converted to cotinine. A small amount o (10%–15%) is excreted in urine, and remaining is further metabolized to other by products.

Cotinine is most commonly used marker to distinguish between tobacco users and non users because of its greater sensitivity and specificity than other biochemical tests. It is stable in body fluids, low plasma protein binding, has a long half-life 15-20 hour.

The presence of cotinine in serum is considered as best marker of tobacco. Another widely used biomarker is urine cotinine level since cotinine concentrations are four to six times higher in urine than that in blood or saliva. In many

nicotine treatment trials, saliva collection is favored over blood and urinary measures as it is easy to obtain and non-invasive.

In the present study also salivary samples are used because it is easy to collect in the dental settings and has an invasive method of collection. The method of collection and the type of specimen impacts the levels of cotinine during detection. The cotinine levels are found to be significantly higher in unstimulated than in stimulated saliva, owing to this we collected unstimulated saliva from the subjects.

The mean salivary cotinine concentration in control group was 6.6498 and in the case group it was 92.8040. Independent t-test showed that there is significant difference in mean cotinine concentration between the groups with a p-value of <0.001 which was consistent with the study done by Renita Lorina Castelino et al[11] and Marieh Honarmand et al[12].

The lowest amount of cotinine concentration measured in th e control group was 1.3ng / ml and the highest was 12.04ng / ml. According to The Society for Research on Nicotine and Tobacco Subcommittee (SRNT) on biochemical verification, the salivary cotinine level in a non-tobacco user is <15ng/ml, so in the present study all the subjects in the control group were having cotinine concentration within the normal limit. But the variation in concentration can be due to difference in food related habits and exposure to environmental tobacco. Nicotine is an found in nightshade family of plants (Solanaceae), it is seen in lower quantities in tomato, potato, eggplant, green pepper, tea leaves etc. People who consumes these foods in larger quantity may have little higher concentration of cotinine.

In the study group the lowest level of cotinine concentration estimated was 82.8ng/ml and highest level estimated was 100ng/ml. This variations can be attributed to the time gap between the consumption of tobacco and time of saliva collection as cotinine is getting cleared from the body, and the other factor like nicotine content of the different brands of smokeless tobacco.

The mean duration of tobacco chewing habit in case group was 11.75. The pearson's correlation was used to estimate the correlation between duration of tobacco chewing and cotinine concentration, it showed there is no correlation between the duration of the tobacco chewing and cotinine concentration in saliva, it can be due to its half life ie, 15 to 19 hrs. . It is in consistent with study done by Patel et al[13] and Etter *et al*[14].

The present study did not analyse cotinine concentration in each different brands of the smokeless tobacco products, and also not considered the age and gender of the subjects. It also did not consider the frequency of the tobacco chewing.

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Conflict of Interest: There were no conflicts of interest

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